

U.S.S.N. 09/991,152

Filed: November 16, 2001

RESPONSE TO OFFICE ACTION

The Claims

1. (previously presented) A genetically engineered organism selected from the group consisting of bacteria and plants producing polyhydroxyalkanoate (PHA),
the improvement comprising providing the organism with a transgene encoding an enzyme having the catalytic activity of 3-hydroxyacyl-ACP thioesterase and one or more transgenes encoding enzymes having the catalytic activity of acyl-CoA synthetase or acyl CoA transferase so that medium chain length PHA accumulates through the fatty acid biosynthesis pathway.
2. (cancelled).
3. (previously presented) The organism of claim 1 wherein the acyl-CoA synthetase is 3-hydroxyacyl-CoA synthetase.
4. (previously presented) The organism of claim 1 comprising a transgene alkK encoding an acyl-CoA synthetase.
5. (previously presented) The organism of claim 1 expressing a heterologous 3-hydroxyacyl-CoA synthetase activity.
6. (previously presented) The organism of claim 1 expressing a heterologous 3-hydroxyacyl-CoA synthetase activity.
7. (previously presented) The organism of claim 1 wherein the enzyme is modified to enhance expression in the genetically engineered organism.
8. (previously presented) The organism of claim 1 expressing an enzyme selected from the group consisting of 3-hydroxyacyl-ACP thioesterase, medium chain length PHA synthase, and medium chain length 3-hydroxy fatty acid acyl CoA synthase, wherein the organism is a plant cell, plant tissue, or whole plant.

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9. (original) The organism of claim 8 further expressing selectable marker genes,
wherein the organism is a whole plant.

10. (previously presented) The organism of claim 1 expressing an enzyme selected from
the group consisting of 3-hydroxyacyl-ACP thioesterase, a PHA synthase that incorporates
medium chain length hydroxy acids, and medium chain length 3-hydroxy fatty acid acyl CoA
synthetase, wherein the organism is a bacteria.

11. (original) The organism of claim 8 wherein expression of the transgene is targeted to
a tissue or organelle selected from the group consisting of seeds, leaf, plastids, and peroxisomes.

12. (original) The organism of claim 10 wherein the bacteria is *E. coli* and PHA
accumulates within the bacteria.

13. (previously presented) A method of engineering a PHA biosynthetic pathway in a
transgenic organism selected from the group consisting of bacteria and plants which produce
polyhydroxyalkanoate (PHA),

the improvement comprising providing the organism with one or more constructs
comprising a transgene encoding an enzyme having the catalytic activity of 3-hydroxyacyl-ACP
thioesterase and one or more transgenes encoding enzymes having the catalytic activity of acyl-
CoA synthetase or acyl CoA transferase so that medium chain length PHA accumulates through
the fatty acid biosynthesis pathway.

14. (cancelled).

15. (previously presented) The method of claim 13 wherein the construct comprises a
transgene encoding a 3-hydroxy acyl-CoA synthetase.

16. (original) The method of claim 15 wherein the construct further comprises a
transgene encoding a PHA synthase.

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17. (original) The method of claim 16 wherein the organism is a plant.

18. (original) The method of claim 16 wherein the construct expresses an enzyme selected from the group consisting of 3-hydroxyacyl-ACP thioesterase, medium chain length PHA synthase, and medium chain length 3-hydroxy fatty acid acyl CoA synthase, wherein the organism is a plant cell, plant tissue, or whole plant.

19. (original) The method of claim 16 wherein the construct expresses an enzyme selected from the group consisting of 3-hydroxyacyl-ACP thioesterase, a PHA synthase that incorporates medium chain length hydroxy acids, and medium chain length 3-hydroxy fatty acid acyl CoA synthetase, wherein the organism is a bacteria.

20. (previously presented) A method of making medium chain length PHA comprising growing a transgenic organism selected from the group consisting of bacteria and plants, the organism producing polyhydroxyalkanoate (PHA) and expressing a transgene encoding an enzyme having the catalytic activity of 3-hydroxyacyl-ACP thioesterase and expressing one or more transgenes encoding enzymes having the catalytic activity of acyl-CoA synthetase or acyl CoA transferase, with substrates for fatty acid biosynthesis.

21. (cancelled).

22. (previously presented) The method of claim 20 wherein the acyl-CoA synthetase is 3-hydroxyacyl-CoA synthetase.

23. (previously presented) The method of claim 20 wherein the organism further express a PHA synthase.

24. (previously presented) The method of claim 22 wherein the organism further express a PHA synthase.

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25. (original) The method of claim 24 wherein the organism expresses an enzyme selected from the group consisting of 3-hydroxyacyl-ACP thioesterase, medium chain length PHA synthase, and medium chain length 3-hydroxy fatty acid acyl CoA synthase, wherein the organism is a plant cell, plant tissue, or whole plant.

26. (original) The method of claim 24 wherein the organism expresses an enzyme selected from the group consisting of 3-hydroxyacyl-ACP thioesterase, a PHA synthase that incorporates medium chain length hydroxy acids, and medium chain length 3-hydroxy fatty acid acyl CoA synthetase, wherein the organism is a bacteria.

27-28. (cancelled)

29. (previously presented) The organism of claim 10 wherein the bacteria is *E. coli*, the bacteria expresses 3-hydroxyacyl-ACP thioesterase and wherein 3-hydroxy acids are secreted into the culture medium.

30. (previously presented) The method of claim 13, wherein the bacteria is *E. coli*, the bacteria expresses 3-hydroxyacyl-ACP thioesterase and wherein 3-hydroxy acids are secreted into the culture medium, further comprising collecting the 3-hydroxy acids from the medium.